

Comparative Evaluation of Antimicrobial Effect of Graphene-Coated, Dexamethasone-Coated and Graphene–Dexamethasone Coated PMMA Implants Used for the Rehabilitation of Craniofacial Defects.

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Abstract:

Background: Polymethyl methacrylate (PMMA) is extensively used in craniofacial rehabilitation due to its favourable mechanical properties and cost-effectiveness. However, its biological inertness and susceptibility to microbial colonization increase the risk of postoperative infections and compromise osteointegration. Surface modification strategies have been introduced to overcome these challenges, particularly graphene-based coatings and incorporation of antimicrobial agents such as dexamethasone. **Aim:** To evaluate and compare the antimicrobial efficacy of graphene-coated, dexamethasone-coated, and graphene–dexamethasone coated PMMA discs against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. **Materials and methods:** An in-vitro experimental study was conducted using 270 PMMA discs divided into three experimental coating groups. Standardized microbial lawns of *S. aureus* and *E. coli* were used to assess antibacterial activity via disc diffusion, while colony counts were recorded for *C. albicans*. Zones of inhibition were measured and compared among the groups to determine relative antimicrobial performance. **Results:** Graphene-coated and graphene–dexamethasone coated PMMA discs demonstrated significantly enhanced antimicrobial activity when compared to dexamethasone alone. The combination (graphene–dexamethasone) coating exhibited the largest zones of inhibition and lowest fungal colony counts, indicating superior broad-spectrum efficacy. **Conclusion:** Graphene–dexamethasone surface-modified PMMA discs show promising antimicrobial potential, outperforming graphene or dexamethasone coatings individually.

Keywords: Antimicrobial activity, craniofacial reconstruction, dexamethasone coating, PMMA implants

Introduction:

Polymethyl methacrylate (PMMA) remains one of the most extensively used materials in cranioplasty and craniofacial reconstruction owing to its favourable mechanical properties, cost-effectiveness, ease of manipulation, and chemical stability.^[1] Despite these advantages, PMMA is biologically inert and does not integrate well with surrounding tissues, which can compromise long-term outcomes in craniofacial rehabilitation.^[2-5] Its limited osteointegration contributes to poor bone healing, implant loosening, and delayed rehabilitation. Another major concern is PMMA's inability to resist microbial adhesion, making it susceptible to colonization by pathogenic organisms such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida*

albicans. These microorganisms readily form biofilms on implant surfaces, rendering them highly resistant to systemic antibiotics and host immune responses, thereby increasing the risk of postoperative infections and implant failure. [6,7]

To address these limitations, several surface enhancement strategies have been explored. Graphene and its derivatives, including graphene oxide, possess strong antimicrobial properties due to their sharp-edged structure, oxidative stress induction, and ability to inhibit biofilm formation. Dexamethasone, a synthetic glucocorticoid known for its osteogenic properties, may enhance the biological performance of implant surfaces by upregulating genes responsible for osteoblast differentiation. Although dexamethasone alone has limited antimicrobial action, its combination with nanomaterials may offer synergistic benefits.^[8] Other antimicrobial coatings such as silver nanoparticles and antibiotics have been investigated, but concerns regarding resistance and cytotoxicity persist. Considering the need for improved antimicrobial and biological properties in PMMA implants, this study aimed to evaluate the antimicrobial effect of graphene-coated, dexamethasone-coated, and graphene–dexamethasone coated PMMA discs against common pathogens associated with implant-related infections.^[9]

Materials and Methods:

This study was designed as an experimental, quantitative, in-vitro microbiological investigation to assess the antimicrobial activity of coated PMMA discs. A total of 450 PMMA discs were fabricated using DPI clear self-cure acrylic resin. The polymer and monomer were proportioned according to manufacturer recommendations, mixed thoroughly to achieve dough consistency, and packed into a customized stainless-steel mold with standardized dimensions of 10 mm diameter and 3 mm thickness. After polymerization, the discs were removed, washed with 0.5% sodium hypochlorite, and finished. Surface polishing was intentionally avoided to preserve micro-roughness and enhance coating adhesion.

Of the 450 discs prepared, 270 were selected and divided into three groups of 90 each for coating. (Table 1) One group was spray-coated with 1% graphene oxide suspension using an ultrasonic spray coater and subsequently baked at 80°C to stabilize the coating. The second group was coated with dexamethasone sodium phosphate in a similar manner and baked at the same temperature. The third group received a combined graphene–dexamethasone coating, followed by identical baking conditions. All coating procedures were performed under sterile laboratory conditions.

Standard laboratory strains of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* were used. Microbial suspensions were adjusted to the 0.5 McFarland standard (approximately $1-2 \times 10^8$ CFU/mL). [10] Using sterile cotton swabs, uniform microbial lawns were prepared by streaking *S. aureus* and *E. coli* on blood agar plates and *C. albicans* on nutrient agar plates. After allowing the plates to dry, coated PMMA discs were placed equidistantly on the agar surfaces using sterile forceps. The plates inoculated with bacterial species were incubated at 37°C for 18–24 hours, whereas those containing *C. albicans* were incubated at 30–35°C for 24–48 hours.

Following incubation, the antimicrobial activity was assessed. For *S. aureus* and *E. coli*, the diameter of the zone of inhibition surrounding each disc was measured in millimetres. (Image

1) For *C. albicans*, colony counts were recorded in the area surrounding each disc. Plates showing contamination, overlapping zones, or inconsistent lawn formation were excluded. The final data were used to compare the antimicrobial efficacy of the three coating types.

Results:

The antimicrobial efficacy of the coated PMMA implant specimens was evaluated against *E. coli*, *C. albicans*, and *S. aureus*. Three coating groups were tested: A3 (Graphene), B3 (Dexamethasone), and C3 (Dexamethasone + Graphene). The results demonstrated substantial variation in the antimicrobial performance among the groups, with the combination coating (C3) consistently showing superior activity across all microorganisms tested. The one-way ANOVA Output using GraphPad compare the means of three or more independent samples (treatments) simultaneously (<https://www.graphpad.com/features/prism-anova>) FOR *E. coli* Null Hypothesis(H_0): All groups have the same antimicrobial Activity for *E. coli* Alternative Hypothesis (H_1): At least one group has a significantly different antimicrobial Activity for *E. coli* (Table: 2) There is a statistically significant difference in between antimicrobial activity the coating groups. A high F-value and low p-value for *E. coli* would confirm that C3 has significantly better antibacterial activity than A3 and B3. (Table 3) Null Hypothesis(H_0): All groups have the same antimicrobial Activity for *C. albicans* Alternative Hypothesis (H_1): At least one group has a significantly different antimicrobial Activity for *C. albicans*. There is a statistically significant difference in between antimicrobial activity the coating groups. A high F-value and low p-value for *C. albicans* would confirm that C3 has significantly better antibacterial activity than A3 and B3. (Table 4) Null Hypothesis(H_0): All groups have the same antimicrobial Activity for *S. aureus* Alternative Hypothesis (H_1): At least one group has a significantly different antimicrobial Activity for *S. aureus*. There is a statistically significant difference in between antimicrobial activity the coating groups. A high F-value and low p-value for *S. aureus* would confirm that C3 has significantly better antibacterial activity than A3 and B3 (Table 5)

Against *E. coli*, the mean zone of inhibition for A3 and B3 ranged between 12–15 mm, whereas C3 exhibited a significantly higher inhibition zone, ranging between 17–20 mm. Similarly, for *S. aureus*, both A3 and B3 produced inhibition zones of 12–15 mm, but C3 showed a markedly larger zone of 17–20 mm, indicating improved antibacterial effects. In the case of *C. albicans*, the colony counts differed dramatically between the groups. A3 showed 35–50 colonies, B3 exhibited the highest count (100–150 colonies), while C3 demonstrated an extremely low fungal colonization of just 4–5 colonies, indicating excellent antifungal efficacy.

One-way ANOVA was performed to compare the antimicrobial activity among the three groups for each microorganism. For *E. coli*, the ANOVA results revealed a highly significant difference between the groups ($F = 32.49$, $p < 0.05$), leading to rejection of the null hypothesis. This indicates that at least one group demonstrated significantly different antibacterial behavior, with post-test comparisons suggesting C3 as the most effective coating. For *C. albicans*, the differences were even more pronounced ($F = 156.22$, $p < 0.05$). The extremely low colony count in the C3 group, compared with the significantly higher counts in A3 and especially B3, confirmed the superior antifungal action of the composite coating. Similarly, for *S. aureus*, the

ANOVA demonstrated significant variation among groups ($F = 33.12$, $p < 0.05$), again establishing C3 as the most potent antibacterial agent of the three.

Graphical analysis further supported these findings. The zone of inhibition graph for *E. coli* (Graph 1) showed that C3 produced the largest mean inhibition zone (~18.4 mm) with narrow error bars, indicating both high antimicrobial potency and reliability of results. A3 and B3 displayed moderate and nearly identical inhibition zones with comparatively low variability. The colony count graph for *C. albicans* (Graph 2) illustrated that C3 resulted in the fewest colonies (~4.4), with minimal standard deviation, confirming its strong and consistent antifungal activity. B3 exhibited the poorest performance with the largest colony counts and wide variability, while A3 demonstrated moderate but stable antifungal action. For *S. aureus* (Graph 3), C3 again showed the highest mean inhibition zone (~18.2 mm), outperforming A3 and B3, both of which demonstrated moderate antimicrobial effects with small standard deviations.

Overall, the results clearly establish that the C3 coating (Dexamethasone + Graphene) offers superior antimicrobial performance compared with the individual coatings (A3 and B3). This group consistently demonstrated the largest zones of inhibition for bacterial strains and the lowest colony count for *C. albicans*, along with low standard deviation values across all tests. (Graph 4) These findings strongly support the hypothesis of a synergistic antimicrobial effect when Dexamethasone and Graphene are combined, suggesting their promising potential for enhancing the antimicrobial properties of PMMA implant surfaces

Discussion:

The present study evaluated the antimicrobial performance of PMMA discs coated with Graphene (A3), Dexamethasone (B3), and their combination (C3), against three clinically significant pathogens: *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Across all microbial assays, the C3 coating consistently demonstrated superior antimicrobial efficacy, supporting the hypothesis of a synergistic interaction between Dexamethasone and Graphene when used together as a composite surface coating. [11-14]

In the case of *S. aureus*, the C3-coated discs exhibited the widest zone of inhibition (~18.2 mm), significantly outperforming both A3 and B3. The low standard deviation observed across all groups confirms that the results are both reliable and reproducible. [15] Considering the well-known role of *S. aureus* in implant-associated infections, these findings suggest that the Dexamethasone–Graphene coating may effectively reduce early bacterial colonization on PMMA implant surfaces. Although A3 and B3 exhibited moderate antibacterial activity, their inhibitory zones remained substantially lower than C3, highlighting the enhanced antimicrobial potential of the combined coating. [16,17]

A similar trend was observed in the analysis of *E. coli*, where the C3 group again produced the largest mean inhibition zone (~18.4 mm). The minimal variability among replicates indicates reproducibility of the antimicrobial effect and reinforces the broad-spectrum action of the composite coating. [18] The enhanced efficacy against Gram-negative bacteria may be attributed to the membrane-disruptive and oxidative stress-inducing properties of Graphene, combined

with the inflammation-modulating effect of Dexamethasone, which likely reduces microbial adherence and persistence.^[19-23]

The antifungal evaluation against *C. albicans* further emphasized the superiority of the C3 coating. While A3 demonstrated moderate antifungal activity and B3 showed poor and highly variable results, C3 produced the lowest colony count (~4.4 colonies) with exceptionally low standard deviation. These findings are particularly important because fungal colonization remains a major risk factor in implant-related infections, especially in immunocompromised individuals. The dramatic reduction in fungal growth achieved by the C3 group highlights the potential of such composite coatings in preventing biofilm formation and improving the long-term safety of PMMA implants.^[24]

Statistical analysis using one-way ANOVA further confirmed the significance of these results. For all three microorganisms, the F-values were markedly high and p-values < 0.05, indicating statistically significant differences among groups. Post hoc Tukey's tests validated that the C3 group was significantly more effective than both A3 and B3, while differences between A3 and B3 were mostly non-significant. This confirms that the enhanced antimicrobial action of the Dexamethasone–Graphene coating is not incidental but statistically validated.^[25]

From a clinical perspective, the consistent and superior performance of the C3 coating, coupled with its minimal standard deviation, highlights both its potency and reliability — essential factors in biomedical applications. Graphene has been reported in earlier studies to exert its antimicrobial effects through membrane disruption, protein adsorption, and induction of oxidative stress, while Dexamethasone modulates inflammation and reduces microbial persistence within tissue microenvironments. Their combined application in this study appears to amplify these effects, offering a multifunctional coating capable of resisting microbial colonization while supporting a more favourable healing environment. These attributes enhance its translational potential for use in craniofacial, orthopedic, and other biomedical implants where infection control is of paramount importance.

The findings strongly support the adoption of Dexamethasone–Graphene composite coatings as a promising approach for improving the antimicrobial performance of PMMA surfaces. By providing broad-spectrum, reproducible, and high-intensity protection against bacterial and fungal pathogens, this coating strategy may contribute significantly to reducing postoperative infections, enhancing implant longevity, and improving patient outcomes.

Conclusion:

The combination coating of Dexamethasone and Graphene (C3) demonstrated superior and consistent antimicrobial performance against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, as evidenced by the largest zones of inhibition and the lowest colony count with minimal standard deviation across all measurements. These findings confirm that the composite coating not only enhances antimicrobial efficacy but also ensures reproducibility — a key requirement for successful clinical application on implant surfaces. The synergistic mechanisms underlying this enhanced performance can be attributed to Graphene's membrane-disruptive and oxidative effects, together with Dexamethasone's ability to modulate local inflammation and reduce microbial persistence. The statistically significant differences

between C3 and the other groups, validated through one-way ANOVA and Tukey's post hoc tests, further substantiate the biological and clinical relevance of these results. In conclusion, the Dexamethasone–Graphene coating offers a promising dual-functional strategy with both antimicrobial and bioactive properties, supporting its potential implementation in craniofacial, orthopedic, and other PMMA-based implants to effectively reduce infection risk and improve clinical outcomes.

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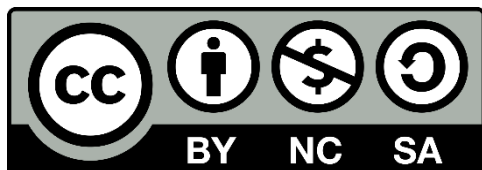
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